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SEQUENCE LISTING

<110> University of Wales, Bangor

Trwyn Ltd

<120> Improvements In and Relating to Biosensors

<130> BA/SLH/Y1861

<160> 9

<170> PatentIn version 3.1

<210> 1

<211> 654

<212> DNA

<213> Escherichia coli K12

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accaactccc agccgtggca ttttattggt gccagcacgg aagaaggtaa agcgcgtggt 180
gccaaatccg ctgccggtaa ttacgtgttc aacgagcgta aaatgcttga tgcctcgcac 240
gtcgtggtgt tctgtgcaaa aaccgcgatg gacgatgtct ggctgaagct ggttggtgac 300
caggaagatg ccgatggccg ctttgccacg ccggaagcga aagccgcgaa cgataaaggt 360
cgcaagttct tcgctgatat gcaccgtaaa gatctgcatg atgatgcaga gtggatggca 420
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gcggtaccca tcgaaggttt tgacgccgcc atcctcgatg cagaatttgg tctgaaagag 540
aaaggctaca ccagtctggt ggttggtccg gtaggtcatc acagcgttga agattttaac 600
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<210> 2

<211> 826

<212> DNA

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<213> *Pseudomonas putida* JLR11

<400> 2

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agcgatcttc ctgtggatga gcagatgctg agctggggcga tcgcgggcggc ccagtcagcc	180
tcgacttcct cgaacctgca agcttgagac gtgttcgccc tgcgggatcg cgagcgtctc	240
gcgaggcttg cccgactgtc cggttaaccag cgccatgtcg agcaggcacc gctgttcctg	300
gtctggctcg tggactggtc acgcctacgc cgactagcca gaacccttca ggcaccgact	360
gcaggtatcg actatttaga aagctacacc gtcggtgttg tagatgcagc tctggccgct	420
cagaacgccg cactagcttt cgaggcccaa ggactgggaa tcgtttacat cggcggaatg	480
cgcaaccacc cggaagcgat gtccgaggag cttggcctgc caaacgacac ttctgctgta	540
tttggcatgt gcgtcgggtc tcccgatccg gcacagcccg ccgagatcaa gccacgcctg	600
gcgcaatcag tgggtgcttca ccgtgagcgc tatgaggcca ccgaggcaga ggcggtttca	660
gttgctgcct atgaccgaag gatgagcgc ttccaacatc gtcaacaacg cgaaaaccgt	720
tcctgggtcca gccaggccgt ggaacgtgta aaaggagcgg attcactgag cggaagacac	780
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<210> 3

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<212> DNA

<213> *Escherichia coli* K12 nfnB in pET-28(a)(+); pMKS2

<220>

<221> CDS

<222> (88)..(858)

<223> Coding sequence for nfnB gene

<220>

<221> misc_feature

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<222> (250)..(267)

<223> Cys tags

<220>

<221> misc_feature

<222> (160)..(177)

<223> His tags

<220>

<221> misc_feature

<222> (268)..(285)

<223> primer

<220>

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<222> (996)..(1010)

<223> primer

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tgtttaactt taagaaggag atatacc atg ggc agc agc cat cat cat cat cat 114
 Met Gly Ser Ser His His His His His
 1 5

cac agc agc ggc ctg gtg ccg cgc ggc agc cat atg gct agc atg act 162
 His Ser Ser Gly Leu Val Pro Arg Gly Ser His Met Ala Ser Met Thr
 10 15 20 25

ggt gga cag caa atg ggt cgc gga tcc tgt tgc tgt tgc tgt tgc gat 210
 Gly Gly Gln Gln Met Gly Arg Gly Ser Cys Cys Cys Cys Cys Cys Asp
 30 35 40

atc att tct gtc gcc tta aag cgt cat tcc act aag gca ttt gat gcc 258
 Ile Ile Ser Val Ala Leu Lys Arg His Ser Thr Lys Ala Phe Asp Ala
 45 50 55

agc aaa aaa ctt acc ccg gaa cag gcc gag cag atc aaa acg cta ctg 306
 Ser Lys Lys Leu Thr Pro Glu Gln Ala Glu Gln Ile Lys Thr Leu Leu
 60 65 70

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caa tac agc cca tcc agc acc aac tcc cag ccg tgg cat ttt att gtt	354
Gln Tyr Ser Pro Ser Ser Thr Asn Ser Gln Pro Trp His Phe Ile Val	
75 80 85	
gcc agc acg gaa gaa ggt aaa gcg cgt gtt gcc aaa tcc gct gcc ggt	402
Ala Ser Thr Glu Glu Gly Lys Ala Arg Val Ala Lys Ser Ala Ala Gly	
90 95 100 105	
aat tac gtg ttc aac gag cgt aaa atg ctt gat gcc tcg cac gtc gtg	450
Asn Tyr Val Phe Asn Glu Arg Lys Met Leu Asp Ala Ser His Val Val	
110 115 120	
gtg ttc tgt gca aaa acc gcg atg gac gat gtc tgg ctg aag ctg gtt	498
Val Phe Cys Ala Lys Thr Ala Met Asp Asp Val Trp Leu Lys Leu Val	
125 130 135	
gtt gac cag gaa gat gcc gat ggc cgc ttt gcc acg ccg gaa gcg aaa	546
Val Asp Gln Glu Asp Ala Asp Gly Arg Phe Ala Thr Pro Glu Ala Lys	
140 145 150	
gcc gcg aac gat aaa ggt cgc aag ttc ttc gct gat atg cac cgt aaa	594
Ala Ala Asn Asp Lys Gly Arg Lys Phe Phe Ala Asp Met His Arg Lys	
155 160 165	
gat ctg cat gat gat gca gag tgg atg gca aaa cag gtt tat ctc aac	642
Asp Leu His Asp Asp Ala Glu Trp Met Ala Lys Gln Val Tyr Leu Asn	
170 175 180 185	
gtc ggt aac ttc ctg ctc ggc gtg gcg gct ctg ggt ctg gac gcg gta	690
Val Gly Asn Phe Leu Leu Gly Val Ala Ala Leu Gly Leu Asp Ala Val	
190 195 200	
ccc atc gaa ggt ttt gac gcc gcc atc ctc gat gca gaa ttt ggt ctg	738
Pro Ile Glu Gly Phe Asp Ala Ala Ile Leu Asp Ala Glu Phe Gly Leu	
205 210 215	
aaa gag aaa ggc tac acc agt ctg gtg gtt gtt ccg gta ggt cat cac	786
Lys Glu Lys Gly Tyr Thr Ser Leu Val Val Val Pro Val Gly His His	
220 225 230	
agc gtt gaa gat ttt aac gct acg ctg ccg aaa tct cgt ctg ccg caa	834
Ser Val Glu Asp Phe Asn Ala Thr Leu Pro Lys Ser Arg Leu Pro Gln	
235 240 245	
aac atc acc tta acc gaa gtg taa ttctctcttg ccgggcatct gcccggtat	888
Asn Ile Thr Leu Thr Glu Val	
250 255	
ttctctcag attctcctga ttgcataac cctgtttcag caagcttcgt catcataggc	948
tgctgttgaa gcttgccggcc gactcgagc accaccacca ccaccactga gatccggctg	1008
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<212> PRT

<213> Escherichia coli K12 nfnB in pET-28(a)(+); pMKS2

<220>

<221> misc_feature

<222> (250)..(267)

<223> Cys tags

<220>

<221> misc_feature

<222> (160)..(177)

<223> His tags

<220>

<221> misc_feature

<222> (268)..(285)

<223> primer

<220>

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<222> (996)..(1010)

<223> primer

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			20					25					30		

Gly	Ser	Cys	Cys	Cys	Cys	Cys	Cys	Asp	Ile	Ile	Ser	Val	Ala	Leu	Lys
		35					40					45			

Arg	His	Ser	Thr	Lys	Ala	Phe	Asp	Ala	Ser	Lys	Lys	Leu	Thr	Pro	Glu
	50					55					60				

Gln	Ala	Glu	Gln	Ile	Lys	Thr	Leu	Leu	Gln	Tyr	Ser	Pro	Ser	Ser	Thr
65					70					75					80

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Asn Ser Gln Pro Trp His Phe Ile Val Ala Ser Thr Glu Glu Gly Lys
85 90 95

Ala Arg Val Ala Lys Ser Ala Ala Gly Asn Tyr Val Phe Asn Glu Arg
100 105 110

Lys Met Leu Asp Ala Ser His Val Val Val Phe Cys Ala Lys Thr Ala
115 120 125

Met Asp Asp Val Trp Leu Lys Leu Val Val Asp Gln Glu Asp Ala Asp
130 135 140

Gly Arg Phe Ala Thr Pro Glu Ala Lys Ala Ala Asn Asp Lys Gly Arg
145 150 155 160

Lys Phe Phe Ala Asp Met His Arg Lys Asp Leu His Asp Asp Ala Glu
165 170 175

Trp Met Ala Lys Gln Val Tyr Leu Asn Val Gly Asn Phe Leu Leu Gly
180 185 190

Val Ala Ala Leu Gly Leu Asp Ala Val Pro Ile Glu Gly Phe Asp Ala
195 200 205

Ala Ile Leu Asp Ala Glu Phe Gly Leu Lys Glu Lys Gly Tyr Thr Ser
210 215 220

Leu Val Val Val Pro Val Gly His His Ser Val Glu Asp Phe Asn Ala
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Thr Leu Pro Lys Ser Arg Leu Pro Gln Asn Ile Thr Leu Thr Glu Val
245 250 255

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<212> DNA

<213> Pseudomonas putida JLR11 prnB in pET-28(a)(+) ; pKMS6

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<221> CDS

<222> (88)..(1029)

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<220>

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<222> (190)..(225)

<223> primer

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<222> (190)..(207)

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<221> misc_feature

<222> (936)..(956)

<223> primer

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tgtttaactt taagaaggag atataacc atg ggc agc agc cat cat cat cat cat 114

Met Gly Ser Ser His His His His His
1 5cac agc agc ggc ctg gtg ccg cgc ggc agc cat atg gct agc atg act 162
His Ser Ser Gly Leu Val Pro Arg Gly Ser His Met Ala Ser Met Thr
10 15 20 25ggt gga cag caa atg ggt cgc gga tcc tgt tgc tgt tgc tgt tgc agc 210
Gly Gly Gln Gln Met Gly Arg Gly Ser Cys Cys Cys Cys Cys Cys Ser
30 35 40ctt caa gac gaa gca ctc aaa gcc tgg caa gcc cgt tat ggc gag cca 258
Leu Gln Asp Glu Ala Leu Lys Ala Trp Gln Ala Arg Tyr Gly Glu Pro
45 50 55gct aac tta cct gct gcc gac acc gtg atc gcg cag atg ttg cag cat 306
Ala Asn Leu Pro Ala Ala Asp Thr Val Ile Ala Gln Met Leu Gln His

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60				65				70								
cga	tca	gta	cgt	gcc	tac	agc	gat	ctt	cct	gtg	gat	gag	cag	atg	ctg	354
Arg	Ser	Val	Arg	Ala	Tyr	Ser	Asp	Leu	Pro	Val	Asp	Glu	Gln	Met	Leu	
	75					80					85					
agc	tgg	gcg	atc	gcg	gcg	gcc	cag	tca	gcc	tcg	act	tcc	tcg	aac	ctg	402
Ser	Trp	Ala	Ile	Ala	Ala	Ala	Gln	Ser	Ala	Ser	Thr	Ser	Ser	Asn	Leu	
90					95					100					105	
caa	gct	tgg	agc	gtg	ctc	gcc	gtg	cgg	gat	cgc	gag	cgt	ctc	gcg	agg	450
Gln	Ala	Trp	Ser	Val	Leu	Ala	Val	Arg	Asp	Arg	Glu	Arg	Leu	Ala	Arg	
				110					115					120		
ctt	gcc	cga	ctg	tcc	ggc	aac	cag	cgc	cat	gtc	gag	cag	gca	ccg	ctg	498
Leu	Ala	Arg	Leu	Ser	Gly	Asn	Gln	Arg	His	Val	Glu	Gln	Ala	Pro	Leu	
			125					130					135			
ttc	ctg	gtc	tgg	ctc	gtg	gac	tgg	tca	cgc	cta	cgc	cga	cta	gcc	aga	546
Phe	Leu	Val	Trp	Leu	Val	Asp	Trp	Ser	Arg	Leu	Arg	Arg	Leu	Ala	Arg	
	140						145					150				
acc	ctt	cag	gca	ccg	act	gca	ggc	atc	gac	tat	tta	gaa	agc	tac	acc	594
Thr	Leu	Gln	Ala	Pro	Thr	Ala	Gly	Ile	Asp	Tyr	Leu	Glu	Ser	Tyr	Thr	
	155					160					165					
gtc	ggc	gtt	gta	gat	gca	gct	ctg	gcc	gct	cag	aac	gcc	gca	cta	gct	642
Val	Gly	Val	Val	Asp	Ala	Ala	Leu	Ala	Ala	Gln	Asn	Ala	Ala	Leu	Ala	
170					175					180					185	
ttc	gag	gcc	caa	gga	ctg	gga	atc	gtt	tac	atc	ggc	gga	atg	cgc	aac	690
Phe	Glu	Ala	Gln	Gly	Leu	Gly	Ile	Val	Tyr	Ile	Gly	Gly	Met	Arg	Asn	
				190					195					200		
cac	ccg	gaa	gcg	atg	tcc	gag	gag	ctt	ggc	ctg	cca	aac	gac	act	ttc	738
His	Pro	Glu	Ala	Met	Ser	Glu	Glu	Leu	Gly	Leu	Pro	Asn	Asp	Thr	Phe	
			205					210					215			
gct	gta	ttt	ggc	atg	tgc	gtc	ggc	cat	ccc	gat	ccg	gca	cag	ccc	gcc	786
Ala	Val	Phe	Gly	Met	Cys	Val	Gly	His	Pro	Asp	Pro	Ala	Gln	Pro	Ala	
		220					225					230				
gag	atc	aag	cca	cgc	ctg	gcg	caa	tca	gtg	gtg	ctt	cac	cgt	gag	cgc	834
Glu	Ile	Lys	Pro	Arg	Leu	Ala	Gln	Ser	Val	Val	Leu	His	Arg	Glu	Arg	
	235					240					245					
tat	gag	gcc	acc	gag	gca	gag	gcg	gtt	tca	gtt	gct	gcc	tat	gac	cga	882
Tyr	Glu	Ala	Thr	Glu	Ala	Glu	Ala	Val	Ser	Val	Ala	Ala	Tyr	Asp	Arg	
250					255					260					265	
agg	atg	agc	gac	ttc	caa	cat	cgt	caa	caa	cgc	gaa	aac	cgt	tcc	tgg	930
Arg	Met	Ser	Asp	Phe	Gln	His	Arg	Gln	Gln	Arg	Glu	Asn	Arg	Ser	Trp	
				270					275					280		
tcc	agc	cag	gcc	gtg	gaa	cgt	gta	aaa	gga	gcg	gat	tca	ctg	agc	gga	978
Ser	Ser	Gln	Ala	Val	Glu	Arg	Val	Lys	Gly	Ala	Asp	Ser	Leu	Ser	Gly	
			285					290					295			
aga	cac	cgc	ttg	cga	gat	gca	tta	aac	acc	cta	ggt	ttc	ggc	ctg	cgc	1026

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Arg His Arg Leu Arg Asp Ala Leu Asn Thr Leu Gly Phe Gly Leu Arg
 300 305 310

tga gatagtgaga tatcccatgc ctattcccg cgcctgaac cggagcacta 1079
 atacctggca actttgcttg agctccgtcg acaagcttgc ggccgcactc gagcaccacc 1139
 accaccacca ctgagatccg gctgctaaca aagcccga aa ggaagctgag ttggctgctg 1199
 ccaccgctga gcaataacta gc 1221

<210> 6

<211> 313

<212> PRT

<213> Pseudomonas putida JLR11 prnB in pET-28(a)(+) ; pKMS6

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<222> (190)..(225)

<223> primer

<220>

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<222> (190)..(207)

<223> cys tag

<220>

<221> misc_feature

<222> (936)..(956)

<223> primer

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Met Gly Ser Ser His His His His His His Ser Ser Gly Leu Val Pro
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Arg Gly Ser His Met Ala Ser Met Thr Gly Gly Gln Gln Met Gly Arg
 20 25 30

Gly Ser Cys Cys Cys Cys Cys Cys Ser Leu Gln Asp Glu Ala Leu Lys
 35 40 45

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Ala Trp Gln Ala Arg Tyr Gly Glu Pro Ala Asn Leu Pro Ala Ala Asp
50 55 60

Thr Val Ile Ala Gln Met Leu Gln His Arg Ser Val Arg Ala Tyr Ser
65 70 75 80

Asp Leu Pro Val Asp Glu Gln Met Leu Ser Trp Ala Ile Ala Ala
85 90 95

Gln Ser Ala Ser Thr Ser Ser Asn Leu Gln Ala Trp Ser Val Leu Ala
100 105 110

Val Arg Asp Arg Glu Arg Leu Ala Arg Leu Ala Arg Leu Ser Gly Asn
115 120 125

Gln Arg His Val Glu Gln Ala Pro Leu Phe Leu Val Trp Leu Val Asp
130 135 140

Trp Ser Arg Leu Arg Arg Leu Ala Arg Thr Leu Gln Ala Pro Thr Ala
145 150 155 160

Gly Ile Asp Tyr Leu Glu Ser Tyr Thr Val Gly Val Val Asp Ala Ala
165 170 175

Leu Ala Ala Gln Asn Ala Ala Leu Ala Phe Glu Ala Gln Gly Leu Gly
180 185 190

Ile Val Tyr Ile Gly Gly Met Arg Asn His Pro Glu Ala Met Ser Glu
195 200 205

Glu Leu Gly Leu Pro Asn Asp Thr Phe Ala Val Phe Gly Met Cys Val
210 215 220

Gly His Pro Asp Pro Ala Gln Pro Ala Glu Ile Lys Pro Arg Leu Ala
225 230 235 240

Gln Ser Val Val Leu His Arg Glu Arg Tyr Glu Ala Thr Glu Ala Glu
245 250 255

Ala Val Ser Val Ala Ala Tyr Asp Arg Arg Met Ser Asp Phe Gln His
260 265 270

Arg Gln Gln Arg Glu Asn Arg Ser Trp Ser Ser Gln Ala Val Glu Arg
275 280 285

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Val Lys Gly Ala Asp Ser Leu Ser Gly Arg His Arg Leu Arg Asp Ala
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Leu Asn Thr Leu Gly Phe Gly Leu Arg
305 310

<210> 7

<211> 24

<212> DNA

<213> Escherichia coli

<400> 7

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24

<210> 8

<211> 27

<212> DNA

<213> Escherichia coli

<400> 8

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27

<210> 9

<211> 42

<212> DNA

<213> Artificial Sequence

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<223> Primer consisting of nfnB gene primer shown in SEQ ID4 with an additional 6 cysteine codons

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42